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CRANIAL OSTEOLOGY OF THE SAND GOBY *NEOGOBIUS FLUVIATILIS* (PALLAS, 1881) FROM THE RIVER SAVA (SERBIA, YUGOSLAVIA)

P. D. SIMONOVIC and V. P. NIKOLIC

Institute of Zoology, Faculty of Biology, University of Belgrade, Studentski trg 16, 11001 Belgrade, Yugoslavia

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Abstract

The intrapopulation variability of the sand goby *Neogobius fluviatilis* (PALLAS, 1811) from the River Sava mostly corresponds to the species intraspecific variability reported previously. Certain characters continuously increase through four examined size classes, while others increase most intensively in the largest size class, whereas the size of the suspensorial opening varies significantly, but irregularly. Allometry was not detected within the examined size range of sand gobies. There were no significant morphological differences between successive size classes The influence of size on shape was negligible. The most variable trait, representing the metapterygoid process, varied stochastically, thus implying that it is of low interest for phylogenetic considerations. Other variable traits mostly describe elements of both upper and lower jaws and gill cover. The changes detected in skull elements (sphenotic and pterotic) are not strongly expressed, due to their small participation in the overall variability of the sample. It seems that gobies over 11 cm in total length are more diverse morphologically than those below that size.

Key words: sand goby, cranial osteology, morphology, intrapopulation variability.

Introduction

Ponto-Caspian gobiids, of the genera *Neogobius* ILJIN 1927, *Proterorhinus* SMITH 1899, *Mesogobius* BLEEKER 1874, *Knipowitchia* ILJIN 1927, *Benthophilodes* BELING and ILJIN 1927 and *Benthophilus* EICHWALD 1831, have been investigated mostly by Russian and Ukrainian ichthyologists. Papers on the genus *Neogobius* by ILJIN (1927, 1949), BERG (1949), PINCHUK (1963, 1976, 1977, 1991) and SVETOVIDOV (1964) dealt mostly with traditional morphological characters of the gobiids, as follows: the seismosensory system of the modified lateral system on the head, the numbers of spines and rays in the fins, the form and structure of the pelvic disc, the form of the dorsal fins, the scale type on the nape and body, the number of scale rows on the body, etc. These reports elucidated the position of the genus *Neogobius*, and enabled most authors to establish the subgeneric division of this genus to the subgenera *Apollonia* ILJIN, 1927; *Ponticola* ILJIN, 1927; *Babka* ILJIN, 1927; and *Neogobius* BERG, 1949. This division was mostly accepted, although some other subgenera were also introduced, e.g.

Eichwaldiella WHITLEY, 1930 and *Chazar* ILJIN in BERG, 1949 (PINCHUK, 1991), comprising the Caspian Sea species the status of which is as yet insufficiently known.

Osteological investigations on the genus *Neogobius* have mostly been limited to reports on the vertebra number. The cranial osteology was not studied until the work of VASILEVA (1988), who investigated most species of this genus.

According to ILJIN (1927, 1949), BERG (1949), PINCHUK (1963, 1976, 1977, 1991) and SVETOVIDOV (1964), the subgenus *Neogobius* is monotypic, with only one species, the sand goby, *N. fluviatilis* PALLAS 1811. Recent papers (VASILEVA, 1988, 1989) on cranial osteology rejected this subgenus, and classified the sand goby together with the round goby *N. melanostomus* (PALLAS, 1811) in the subgenus *Apollonia*.

The intraspecific osteological variability of the sand goby was reported by VASILEVA (1988). That paper dealt with different qualitative character states within particular populations for several traits, as well as with the interpopulation variability for the frequencies of these character states. Several traits, different for particular populations, were also reported as variable regarding the size of the investigated specimens, and the sexual dimorphism was also quoted for some characters. However, there was no detailed information on the significance of the reported differences. Various qualitative qualifications (e.g. "undoubtedly different" or "differences not great") were given instead. Further, there was no difference in skull structure between the Azov Sea (*N. fluviatilis fluviatilis*) and the Caspian Sea (*N. f. pallasi*) sand gobies. The final conclusion from these investigations was that the "intraspecific variability of the skull of the sand goby is not great, which implies that craniological characteristics of the species are sufficiently compact" (VASILEVA, 1988).

The morphometric investigation of the cranial osteology of a particular species of the genus *Neogobius* has the aim of contributing to their classification. This aim is of further interest as regards the western distribution area of the River Sava sand goby population, which is advatageous for a comparison with populations from the distribution centre of this species. Therefore, an investigation of the intraspecific variability of the sand goby is useful for making decision concerning the use of particular characters in the phylogenetic analysis of the genus.

Material and methods

Specimens for osteological analysis were caught by angling (hook size 12-16), mostly onshore, at a depth of 0.5-1.5 m, on the River Sava left bank, app. 1.5-2.5 km upstream from its mouth into the River Danube, in the Belgrade area, during 1994. A total of 16 specimens were analyzed. Their sex was not determined. Their total length varied between 95.8 mm and 135.4 mm.

The preparation of skeletons was as follows: skinning, flesh removal by *Dermestes lardarius* (Dermestidae, Coleoptera), a short bleaching (3% H_2O_2) and hot water immersion (for app. 30 minutes, depending on the size of a skeleton) for decomposition of splanchnocranium, shoulder girdle and operculum. 50 osteological characters were measured description and abbreviation of which is summarized in Table 1. They were processed as follows:

By descriptive statistics on indices for the whole data set, in order to allow comparison with the results presented in VASILEVA (1988). Characters on the skull skeleton were indexed vs. the skull base length, as

well as characters of other head skeleton units (suspensorium, jaw apparatus, gill cover and pectoral girdle). Characters of particular bone parts were indexed on the respective bone length.

By unifactorial ANOVA (SOKAL and ROHLF, 1981), on indices for 4 size classes. The first one (1) comprised specimens smaller than 10 cm in total length; the second one (2) contained specimens with a total length between 10 cm and 10.9 cm; the total length range of the third size class (3) specimens was 11-11.9 cm; while specimens of the fourth size class (4) had a total length over 12 cm.

By multivariate methods on the logarithmic-transformed raw data set (SNEATH and SOKAL, 1973), i.e. by sheared principal component analyses on variance-covariance matrix (BOOKSTEIN *et al.*, 1985), and by UPGMA clustering of Manhattan distances between sheared principal component score centroids of size classes. Scores from the covariance matrix analysis served for the calculation of ontogenetic trajectories, according to HUMPHRIES *et al.* (1981), and their multiple comparison between successive size classes by TUKEY'S q-test (ZAR, 1984), whereas loadings from an ordination of the correlation matrix were used for an inspection of allometry, according to JOLICEUR (1963).

Results

The head skeleton includes a moderately high skull, with a straight posterior part of the roof, and a curved descent on the anterior part, from the rear edge of the orbit to the tip of the vomer (Fig. 1). The greatest width of the skull is at the pterotic level, and the smallest at the level of the exoethmoid (Fig. 2). The dental is relatively short and high, especially at its rear end, with no prominent teeth at the rear end of the rather long dental row. The rear, lower part of the articular is low (less than 1/5 of the bone height) and short (less than 2/3 of the bone length). The praemaxillar is relatively short and high. The anterior articulating surface of the palatine is well developed and comprises more than half of the whole bone length. The height of the hyomandibular is approximately equal to its length. The rear, stout part of the praeoperculum is relatively wide, and angle- or almost boomerang-shaped, with a narrow, long "handle". The ventral part of the cleithrum is relatively short and stout (Fig. 3).

A comparison of our results (Table 1) with the data of VASILEVA (1988) revealed that the sand goby from the River Sava is similar to the sand goby from the Azov Sea in Spt, Wpraeop, 12praemax, lhyom, Whyoman and Wcleit; it has bigger values for Hmax, Lforsus, Lorbit, lsubop,wpraeop, lpraeop, Hdent, latric and Hartic; and smaller values for all other characters. In many characters (e.g. Spt, Lforsus, Sarcdent, Lprocsph, Lorbit, Wopec, Wab, Wsubop, lsubop, Wpraeop, wpraeop, lpraeop, Hdent, ldent, latric, hartic, Hpraemax and hcpraemax), the sand goby from the River Sava is more similar to those from the different Caspian Sea tributary populations than to the Azov Sea sand goby.

The variability between the four established size classes (Table 2) was not marked for most of the osteological traits of the cranium. Nevertheless, several characters revealed a continuous increase, e.g. Ssp, Spt, Lprocsph and lsubop. This increase was regular through all examined size clases. A few characters (e.g. Hartic and hartic) varied, i.e. they increased significantly between the largest size classes only, while the previous increase was insignificant. Only one of the characters, i.e. Lforsus, decreased at first, and thereafter increased significantly.

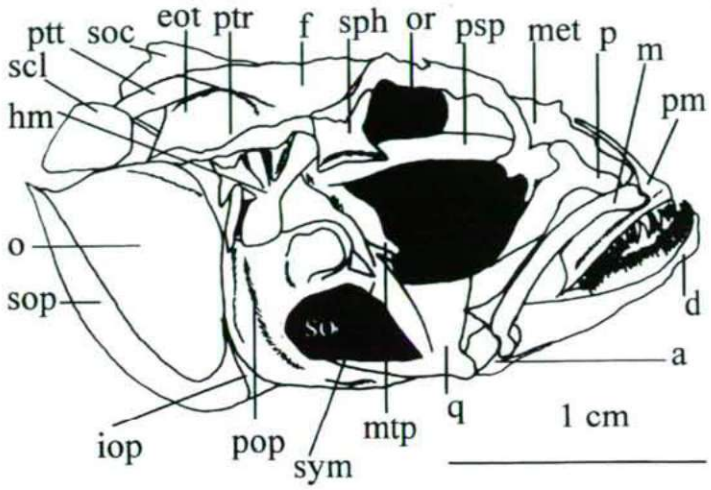

Fig. 1. The cranium of the sand goby, including the operculum and pectoral girdle - a gross view (a: articular; d: dental; eot: epiotic; f: frontal; hm: hyomandibular; iop: interoperculum; m: maxillar; mtp: metapterygoid; o: operculum *s. stricto*; or: orbit; p: palate; pm: praemaxillar; pop: praeoperculum; psp: parasphenoid; ptr: pterotic; ptt: posttemporal; q: quadrate; so: an opening between suspensorium (i.e. quadrate, metapterygoid and symplectic) and praeoperculum; scl: supracleithrum; soc: supraoccipital; sop: suboperculum; sph: sphenotic).

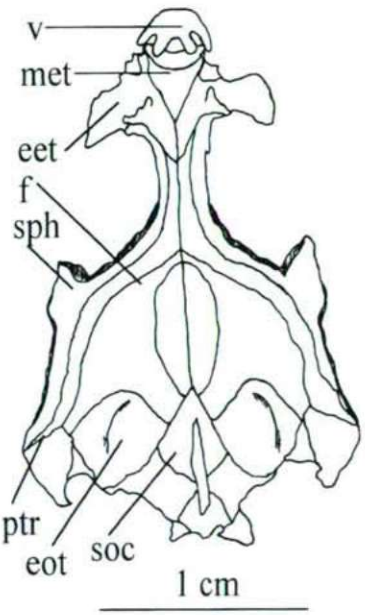

Fig. 2. Dorsal side of the cranium of *Neogobius fluviatilis* (eet: exoethmoid; v: vomer; other symbols as in Fig. 1).

Table 1. Descriptive statistics results (min = minimum; max = maximum; M = mean; s = standard error of mean; CV = variation coefficient) for the osteological characters of *Neogobius fluviatilis* (n = 16).

Character	Description	min	max	M	s	CV
Lcran	skull base length	16.4	22.2	18.9	0.4	9.0
Hmax	maximal height of skull at level of supraoccipital crest	26.6	30.8	28.4	0.3	4.2
Hm	skull height at mesethmoid level	10.7	15.5	13.1	0.3	9.1
Hfr	skull height at frontal level	22.2	25.7	23.8	0.2	3.9
Sprf	skull width at exoethmoid level	28.3	39.1	33.5	0.8	9.0
Ssp	skull width at sphenotic level	47.3	53.6	49.2	0.4	3.5
Spt	maximal skull width at pterotic level	63.7	70.3	66.3	0.5	2.9
Lforsus	greater diagonal width of suspensorial opening	23.7	26.2	25.0	0.2	3.1
Lpraeorb	praeorbital length	13.6	18.0	16.0	0.3	7.3
Sarcdent	width of intact dentary arch at articulare-quadratum joint level	26.8	41.9	33.2	0.9	10.3
Lprocsph	sphenotic extension length	10.0	14.0	11.3	0.3	9.8
Lsphpter	sphenotic-pterotic length	42.3	47.8	44.8	0.3	3.0
Lorbit	diagonal orbit length	31.5	37.5	33.8	0.4	4.5
Loperc	rear opercular edge length	44.6	61.7	49.4	1.1	9.3
Woperc	maximal opercular width	38.0	63.2	52.8	3.5	13.2
Lsubop	subopercular width	55.7	64.0	59.4	0.6	4.1
lsubop	length of lower anterior subopercular extension	37.1	49.6	42.0	0.8	7.2
Wsubop	subopercular width	19.0	34.4	25.0	1.0	16.0
Lpraeop	length of praeopercular	55.0	66.5	59.0	0.8	5.1
lpraeop	length of lower praeopercular extension that joins quadrate	38.5	52.5	45.6	0.9	7.7
Wpraeop	maximal praeopercular width	36.8	48.2	44.0	0.8	7.4
wpraeop	praeopercular width without anterior middle extension	30.9	38.5	35.2	0.5	5.9
Ldent	length of dentary	34.5	49.8	40.0	0.8	8.0
ldent	length of teeth row	58.6	74.4	65.9	1.1	7.0
Hdent	rear maximal height of dentary	37.7	48.3	40.6	0.7	7.1
hdent	height of dentary at level of rear end of teeth row	17.8	25.7	20.9	0.4	8.6
Lartic	maximal length of articular	33.2	40.2	36.4	0.5	5.6
lartic	length of lower palate of articular	52.7	64.9	59.7	0.8	5.7
Hartic	maximal height of articular	38.1	56.3	43.4	1.1	9.7
hartic	height of lower palate of articular	15.5	21.4	18.6	0.4	8.8
Lpraemax	length of praemaxilla	28.3	35.3	30.1	0.5	6.1
l1praemax	length of depressed middle part of praemaxilla ridge	11.1	32.7	16.7	1.3	29.9
l2praemax	length of the elevated rear part of praemaxilla ridge	40.4	51.7	45.4	0.9	8.3
Hpraemax	maximal height of praemaxilla	58.6	68.1	62.8	0.7	4.5
hpraemax	height of praemaxilla articulation surface for joint with skull	27.5	36.7	33.2	0.7	8.0
hcpraemax	height of elevated rear part of praemaxilla ridge	18.3	24.5	21.8	0.4	8.1
Lpalat	length of palatine	23.8	32.6	26.4	0.5	7.9
lpalat	length of palatine front articulation surfaces	58.6	75.6	67.6	1.0	5.9
Lab	length of ventral part of last gill arch	56.0	77.0	63.1	1.3	8.3
Wab	width of ventral part of last gill arch	14.0	21.1	17.8	0.4	9.7
Lcleit	cleithrum length	79.2	90.7	84.5	0.8	3.9
lcleit	length of ventral part of cleithrum	47.0	59.7	51.6	0.7	5.6
Wcleit	cleithrum width	15.1	21.0	17.3	0.4	9.0
Lhyomand	length of hyomandibular, praeopercular extension included	30.0	40.0	32.8	0.6	6.9
lhyomand	length of hyomandibular, without praeopercular extension	104.8	132.3	115.7	1.7	5.8
Whyomand	maximal length of hyomandibular	79.7	109.2	102.1	1.6	6.4
l1/l2ptemp	length of ventral extension of posttemporal	54.0	83.1	68.1	2.1	12.6
Lmaxil	length of maxilla	35.1	41.9	37.2	0.5	5.1
lmaxil	length of front articulation surfaces of maxillar bone	27.0	43.3	35.5	1.3	14.9
Lmpter	length of metapterygoid	20.2	32.1	26.3	0.7	10.7
lmpter	length of lower metapterygoid extension	0.0	14.7	5.5	0.9	63.1

Table 2. Unifactorial ANOVA results for the comparison of particular characters (for indices see Table 1) through size classes (<10 cm; 10-11 cm; 11-12 cm; >12 cm) of *Neogobius fluviatilis* (M = mean; s = standard error of mean; F = F-value; p = probability: *** = 0.01; ** = 0.02; * = 0.05; df = degree of freedom).

Character	<10 cm M ± s n = 4	10-11 cm M ± s n = 4	11-12 cm M ± s n = 4	>12 cm M ± s n = 4	F df = 3	p<
L.tot (mm)	98.60 ± 0.99	105.5 ± 0.51	115.6 ± 1.79	126.8 ± 3.06	51.90	***
Lcran	17.10 ± 0.44	17.98 ± 0.19	19.33 ± 0.60	21.03 ± 0.59	12.30	
Hmax	28.02 ± 0.67	29.36 ± 0.45	28.25 ± 0.90	28.08 ± 0.24	1.06	
Hm	12.57 ± 0.62	13.08 ± 0.43	12.63 ± 0.56	13.94 ± 0.76	1.10	
Hfr	23.54 ± 0.15	23.79 ± 0.48	24.02 ± 0.70	23.90 ± 0.61	0.10	
Sprf	32.04 ± 0.51	31.17 ± 1.76	35.73 ± 1.68	35.13 ± 0.98	2.78	
Ssp	48.40 ± 0.73	47.99 ± 0.37	49.42 ± 0.65	51.17 ± 0.95	4.02	*
Spt	64.45 ± 0.36	65.79 ± 0.77	66.48 ± 0.55	68.54 ± 0.99	5.84	**
Lforsus	25.16 ± 0.37	25.04 ± 0.14	24.08 ± 0.31	25.68 ± 0.31	5.10	**
Lpraeorb	15.74 ± 0.63	15.58 ± 0.82	16.15 ± 0.51	16.49 ± 0.54	0.41	
Sarcdent	30.69 ± 1.33	32.50 ± 1.52	32.53 ± 1.16	36.96 ± 1.73	3.39	
Lprocsph	10.38 ± 0.12	11.13 ± 0.27	11.30 ± 0.53	12.56 ± 0.68	3.88	*
Lsphpter	43.98 ± 0.62	45.48 ± 0.86	45.32 ± 0.56	44.32 ± 0.70	1.12	
Lorbit	34.99 ± 0.62	34.48 ± 1.07	32.71 ± 0.32	33.10 ± 0.56	2.42	
Loperc	51.40 ± 2.75	46.58 ± 0.69	47.80 ± 1.07	51.93 ± 3.43	1.33	
Woperc	50.92 ± 5.16	41.10 ± 12.75	52.71 ± 2.50	52.33 ± 4.00	0.25	
Lsubop	61.41 ± 1.49	57.98 ± 0.85	59.93 ± 1.01	58.14 ± 1.08	2.06	
lsubop	39.23 ± 0.89	40.75 ± 0.60	43.21 ± 1.30	44.90 ± 1.71	4.41	*
Wsubop	22.98 ± 1.71	24.16 ± 2.23	25.87 ± 1.96	27.08 ± 2.54	0.72	
Lpraeop	58.62 ± 0.58	58.15 ± 1.28	57.84 ± 1.00	61.32 ± 2.56	1.07	
lpraeop	47.46 ± 2.03	48.28 ± 1.28	44.30 ± 0.56	42.45 ± 1.76	3.24	
Wpraeop	42.83 ± 1.37	45.19 ± 1.39	44.60 ± 1.67	43.57 ± 2.54	0.34	
wpraeop	33.10 ± 0.91	34.70 ± 0.95	36.25 ± 0.95	36.61 ± 0.69	3.32	
Ldent	38.27 ± 1.29	39.07 ± 0.62	39.93 ± 1.01	42.61 ± 2.66	1.41	
ldent	64.82 ± 3.00	65.62 ± 2.10	64.88 ± 1.56	68.14 ± 3.13	0.38	
Hdent	41.87 ± 2.24	40.99 ± 1.65	38.64 ± 0.37	40.73 ± 1.12	0.82	
hdent	21.93 ± 1.52	19.96 ± 0.74	2.77 ± 0.64	21.12 ± 0.59	0.73	
Lartic	36.07 ± 1.02	37.27 ± 0.71	36.21 ± 1.47	36.00 ± 1.18	0.28	
lartic	61.69 ± 1.53	58.21 ± 0.81	59.23 ± 3.08	59.85 ± 0.77	0.65	
Hartic	42.59 ± 0.79	40.29 ± 1.27	41.98 ± 1.06	48.76 ± 2.56	5.52	**
hartic	17.37 ± 0.61	17.94 ± 0.94	18.65 ± 0.62	20.49 ± 0.32	4.22	*
Lpraemax	29.62 ± 0.83	29.6 ± 0.68	29.53 ± 0.49	31.68 ± 1.42	1.29	
l1praemax	16.45 ± 1.32	15.09 ± 1.31	21.57 ± 3.96	13.69 ± 1.55	2.19	
l2praemax	44.27 ± 1.18	42.29 ± 1.19	47.29 ± 1.77	47.87 ± 2.50	2.25	
Hpraemax	63.68 ± 0.94	62.94 ± 1.48	60.11 ± 0.66	64.45 ± 1.86	2.06	
hpraemax	33.57 ± 1.78	30.99 ± 1.53	34.15 ± 1.19	34.13 ± 0.42	1.28	
hcpraemax	21.15 ± 1.12	22.17 ± 0.96	21.10 ± 1.19	21.73 ± 0.52	0.25	
Lpalat	26.00 ± 0.58	26.70 ± 1.16	25.59 ± 0.61	27.43 ± 1.74	0.52	
lpalat	67.37 ± 0.53	66.46 ± 2.25	69.79 ± 2.25	66.77 ± 2.91	0.48	
Lab	60.25 ± 2.46	62.19 ± 2.24	64.81 ± 1.92	65.01 ± 4.09	0.66	
Wab	17.20 ± 0.64	17.29 ± 1.41	18.34 ± 0.62	18.56 ± 0.88	0.56	
Lcleit	82.09 ± 1.32	82.77 ± 0.64	86.33 ± 1.86	86.94 ± 1.65	2.88	
lcleit	51.17 ± 1.03	51.78 ± 1.71	52.05 ± 2.62	51.41 ± 0.35	0.06	
Wcleit	17.05 ± 0.41	17.14 ± 0.35	16.40 ± 0.65	18.67 ± 1.26	1.60	
Lhyomand	31.85 ± 0.53	32.27 ± 0.58	32.23 ± 0.50	34.79 ± 2.06	1.43	
lhyomand	117.7 ± 3.40	114.3 ± 1.83	114.2 ± 3.48	116.8 ± 5.52	0.23	
Whyomand	103.2 ± 1.48	101.5 ± 1.68	104.6 ± 1.65	99.19 ± 6.63	0.42	
l1/l2ptemp	68.67 ± 5.23	68.84 ± 4.13	67.38 ± 4.01	67.50 ± 6.10	0.02	
Lmaxil	35.94 ± 0.38	36.72 ± 0.53	37.01 ± 0.77	39.09 ± 1.40	2.43	
lmaxil	34.36 ± 2.27	37.00 ± 3.48	37.03 ± 3.35	33.62 ± 2.35	0.37	
Lmpter	25.11 ± 1.64	24.85 ± 1.54	27.06 ± 0.61	28.15 ± 1.60	1.26	
lmpter	6.31 ± 2.93	2.93 ± 1.15	6.14 ± 1.30	6.78 ± 1.26	0.94	

The results of the multivariate analysis demonstrated the negligible influence of the general size component. Neither allometry during the growth ($\chi^2 = 0.300$; df = 51), nor a difference in the calculated ontogenetic trajectories between size classes was detected (Table 3). The highest loading on PC1 ($\lambda_1 = 4.480$; 93.89%), i.e. a size component, was that of lmpter, whereas Woperc had the highest loadings on PC2 ($\lambda_2 = 0.168$; 3.51%) and PC3 ($\lambda_3 = 0.077$; 1.61%), i.e. shape components, both before and after the shearing. Some other characters, e.g. Wsubop, 12praemax, ldent and hdent, loaded PC2 highly, whereas Sarcdent, hartic, lmaxill and Lmpter loaded PC3 (Table 4).

Table 3. Ontogenetic trajectories and their standard error (diagonal) for four size classes of *Neogobius fluviatilis*, and their successive testing by TUKEY'S q test (k = 4; n = 16; below diagonal).

Size class	<10 cm	10-11 cm	11-12 cm	>12 cm
<10 cm	11.55 ± 6.80			
10-11 cm	3.681	-0.47 ± 0.22		
11-12 cm		2.623	14.16 ± 6.26	
>12 cm			1.053	21.66 ± 6.49

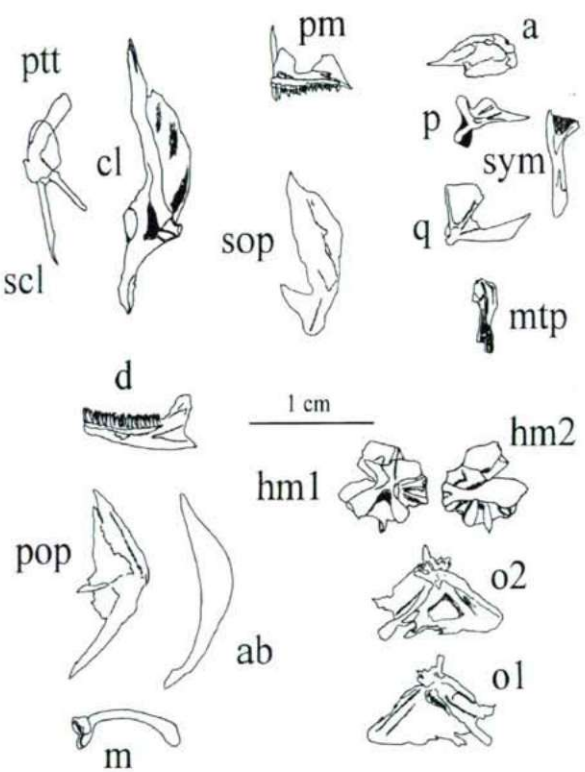

Fig. 3. Bones of the splanchnocranium, operculum *s. lato* and pectoral girdle of *Neogobius fluviatilis* (ab: fifth branchial arch; hm1: inner side, and hm2: outer side of hyomandibular; o1: outer side, and o2: inner side of operculum *s. stricto*; sym: symplectic; other symbols as in Fig. 1).

Table 4. Principal component loadings of particular osteological characters (for legends see Table 1) of *Neogobius fluviatilis* before (PC1-3) and after (H2-3) shear.

Character	PC1	PC2	PC3	H2	H3
Lcran	0.003	0.084	-0.059	0.084	-0.059
Hmax	0.002	0.087	-0.065	0.087	-0.065
Hm	0.008	0.109	-0.079	0.109	-0.079
Hfr	0.006	0.086	-0.059	0.086	-0.059
Sprf	0.006	0.145	-0.027	0.145	-0.027
Ssp	0.005	0.110	-0.064	0.110	-0.064
Spt	0.003	0.112	-0.068	0.112	-0.068
Lforsus	0.004	0.086	-0.063	0.086	-0.063
Lpraeorb	-0.001	0.145	-0.031	0.145	-0.031
Sarcdent	-0.001	0.145	-0.131	0.145	-0.131
Lprocsph	0.003	0.157	-0.084	0.157	-0.084
Lsphpter	0.000	0.092	-0.059	0.092	-0.059
Lorbit	0.005	0.044	-0.074	0.044	-0.074
Loperc	0.005	0.100	-0.059	0.100	-0.059
Woperc	-0.005	0.487	0.847	0.487	0.847
Lsubop	0.002	0.083	-0.041	0.083	-0.041
lsubop	0.004	0.131	-0.077	0.131	-0.077
Wsubop	-0.004	0.199	-0.040	0.199	-0.040
Lpraeop	0.002	0.118	-0.060	0.118	-0.060
lpraeop	-0.002	0.056	-0.030	0.056	-0.030
Wpraeop	-0.003	0.107	-0.073	0.107	-0.073
wpraeop	0.004	0.158	-0.054	0.158	-0.054
Ldent	0.004	0.033	-0.097	0.033	-0.097
ldent	0.006	0.171	-0.081	0.171	-0.081
Hdent	0.004	0.121	-0.096	0.121	-0.096
hdent	0.005	0.167	-0.039	0.167	-0.039
Lartic	0.000	0.088	-0.073	0.088	-0.073
lartic	0.001	0.086	-0.048	0.086	-0.048
Hartic	0.005	0.146	-0.084	0.146	-0.084
hartic	0.011	0.129	-0.119	0.129	-0.119
Lpraemax	0.002	0.129	-0.068	0.129	-0.068
l1praemax	0.016	0.103	0.060	0.103	0.060
l2praemax	0.008	0.172	-0.051	0.172	-0.051
Hpraemax	0.003	0.140	-0.053	0.140	-0.053
hpraemax	0.008	0.139	-0.092	0.139	-0.092
hcpraemax	0.008	0.102	-0.078	0.102	-0.078
Lpalat	-0.003	0.121	-0.076	0.121	-0.076
lpalat	0.003	0.115	-0.056	0.115	-0.056
Lab	0.004	0.134	-0.082	0.134	-0.082
Wab	0.018	0.165	-0.059	0.165	-0.059
Lcleit	0.006	0.109	-0.063	0.109	-0.063
lcleit	0.002	0.117	-0.070	0.117	-0.070
Wcleit	0.003	0.159	-0.087	0.159	-0.087
Lhyomand	0.002	0.125	-0.079	0.125	-0.079
lhyomand	0.005	0.122	-0.088	0.122	-0.088
Whyomand	0.003	0.132	-0.086	0.132	-0.086
l1ptemp	0.002	0.045	-0.090	0.045	-0.090
l2ptemp	0.002	0.079	-0.031	0.079	-0.031
Lmaxil	0.003	0.123	-0.075	0.123	-0.075
lmaxil	-0.001	0.093	-0.138	0.093	-0.138
Lmpter	0.002	0.137	-0.130	0.137	-0.130
lmpter	0.999	-0.020	0.015	-0.020	0.015

The sheared component scores (Fig. 4) and a phenogram of examined size class specimen centroids (Fig. 5), based on the UPGMA clustered Manhattan distances between them (Table 5), revealed the directed morphology formation. The most similar successive size classes were the smallest ones, while the most dissimilar were the largest. Moreover, the dendrogram suggests that all sand gobies over 11 cm in total length are very different from those below that size, i.e. the most similar size classes are the smallest ones.

Table 5. Manhattan distances between sheared score centroids of *Neogobius fluviatilis* size classes.

Size class	<10 cm	10-11 cm	11-12 cm	>12 cm
<10 cm	0.000			
10-11 cm	1.802	0.000		
11-12 cm	4.845	3.043	0.000	
>12 cm	9.009	7.207	4.164	0.000

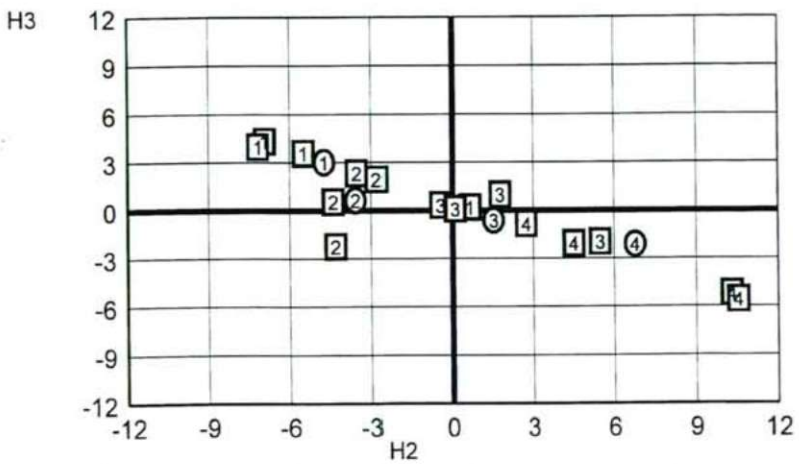

Fig. 4. Sheared principal component (H2 and H3) scores of sand goby specimens (boxes) of particular size classes (1-4) and centroids for size classes (circles).

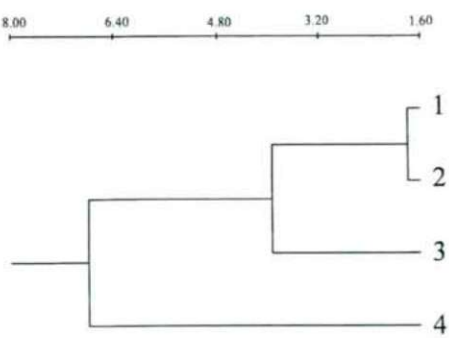

Fig. 5. Dendrogram of size class (1-4) centroids of *Neogobius fluviatilis*.

Discussion

The total lengths of the specimens examined in the paper of VASILEVA (1988) and in the present work indicated that the samples were very similar and thus might be comparable. No data on the distribution of the particular size classes were given in the work of VASILEVA (1988). It must be taken into account that this work included a few large specimens (i.e. four over 12 cm in total length), and this might be the reason for the differences between particular populations that appeared in some features. The description of particular skull bones mostly corresponds to that of VASILEVA (1988). Similar prominent features regarding both visual impression and descriptive statistics of the whole skull and particular bones (i.e. dental, articulare, hyomadibulare and cleithrum) and the ratios of some bone parts were also stressed here. It was suggested that there was no great variability of these traits. The comparison between sand goby populations from the River Sava and the Caspian Sea tributaries and the only specimen from the Azov Sea revealed that the sand gobies were rather similar in many characters. Many differences in examined characters were not significant on ANOVA, and even on pairwise testing, i.e. the approximative pairwise comparison ($M_{i,j} \pm 2s_{i,j}$). However, it seems that the sand gobies from the River Sava are more similar to those from the Caspian Sea tributaries in certain traits than to the specimen from the Azov Sea, and in other traits they are different. This strongly corroborated an inference on the consistent range of variability of this species (VASILEVA, 1988).

The intrapopulation variability between the particular size classes was noteworthy. Although several characters varied significantly, especially those in the largest sand gobies (over 12 cm in total length), they had no outstanding impact on their ontogenetic trajectories, most probably due to the small variability inherent to them. The UPGMA clustering of size class centroids (Fig. 5) and the concordance with the ontogenetic trajectories corroborates an inference on the direct pattern of morphology formation through the examined size classes.

With regard to the facts that both lmpter almost exclusively loaded PC1, and the sheared shape axes (i.e. H2 and H3) did not differ from their precursors (PC2 and PC3), it was evident that the effect of size on the overall morphology of the sand goby was extremely small. This was probably because of the size range of the analyzed specimens. lmpter could be regarded as an extraordinary variable trait, since it had a variation coefficient of 63.1% (Table 1). Its correlation with size seemed remarkable, although descriptive statistics results and ANOVA testing (Tables 1 and 2) did not reveal this character as significant and discriminative for size classes, implyinng that it varied individually and stochastically. It is possible that the relative small lmpter in the 10-10.9 cm size class affected the multivariate analysis strongly by its variation. Thus, this character may be regarded as inappropriate for any discrimination analysis.

The most variable traits related to the jaw apparatus of the splanchnocranium (both dermal and substituent), and to the gill cover. Skull elements (i.e. sphenotic and pterotic) that changed significantly in particular size classes did not participate

markedly in the overall variation, and thus did not load the firs three principal components.

Only one character of 8 reported as noteworthy for variation due to size in sand gobies from the Caspian Sea watershed (VASILEVA, 1988), i.e. Spt, varied similarly as in sand gobies from the River Sava according to unifactorial ANOVA testing. Two characters, Lprocsph and hartic, were prominent by unifactorial ANOVA and PCA, which implies that they are both rather variable and at the same time discriminative for size classes. Thus, it seems that they should be taken into account for further phylogenetic considerations.

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